

Europäisches Patentamt
European Patent Office
Office européen des brevets



(11) EP 0 873 997 A1

(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication
28.10.1998 Bulletin 1998/44

(51) Int Cl.⁶: **C07K 9/00, A61K 38/14**

(21) Application number 98302868.9

(22) Date of filing: 14.04.1998

(84) Designated Contracting States
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC
NL PT SE
Designated Extension States
AL LT LV MK RO SI

(72) Inventors:

- Cooper, Robin David Grey
Indianapolis, Indiana 46220 (US)
- Snyder, Nancy June
Charlottesville, Indiana 46117 (US)

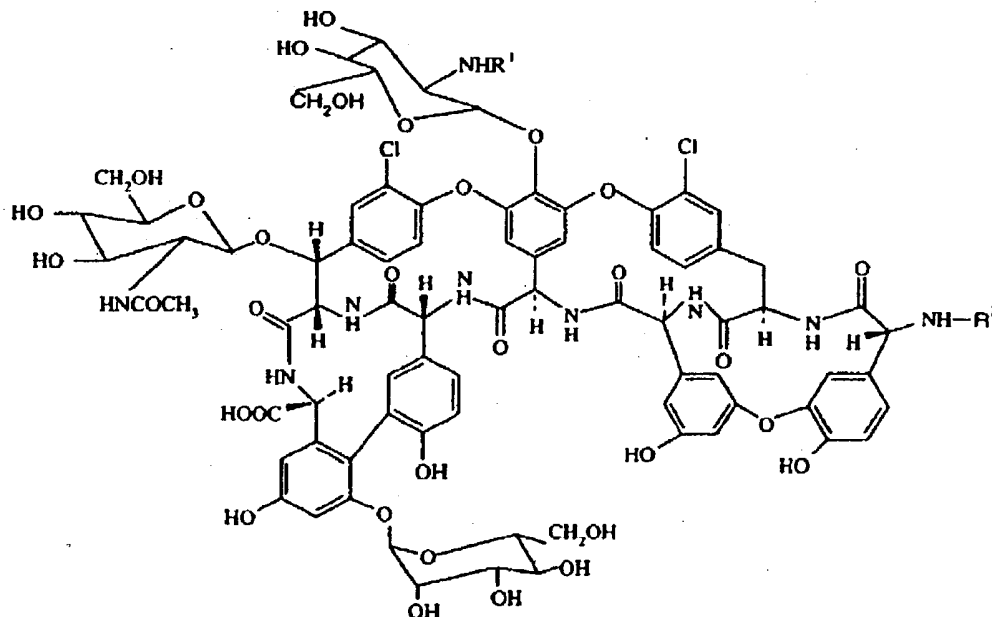
(30) Priority: 17.04.1997 US 42017 P

(74) Representative: Denholm, Anna Marie et al
Eli Lilly and Company Limited,
Lilly Research Center,
Erl Wood Manor
Windlesham, Surrey GU20 6PH (GB)

(71) Applicant: ELI LILLY AND COMPANY
Indianapolis, Indiana 46285 (US)

(54) Teicoplanin derivatives

(57) The present invention is directed to toxoplasmin derivatives of the following general formula:



These derivatives are useful as antibiotics for the control of gram-positive bacteria

EP 0 873 997 A1

Printed by Jouve, 75001 PARIS (FR)

EP 0 873 997 A1

Description

CROSS REFERENCE

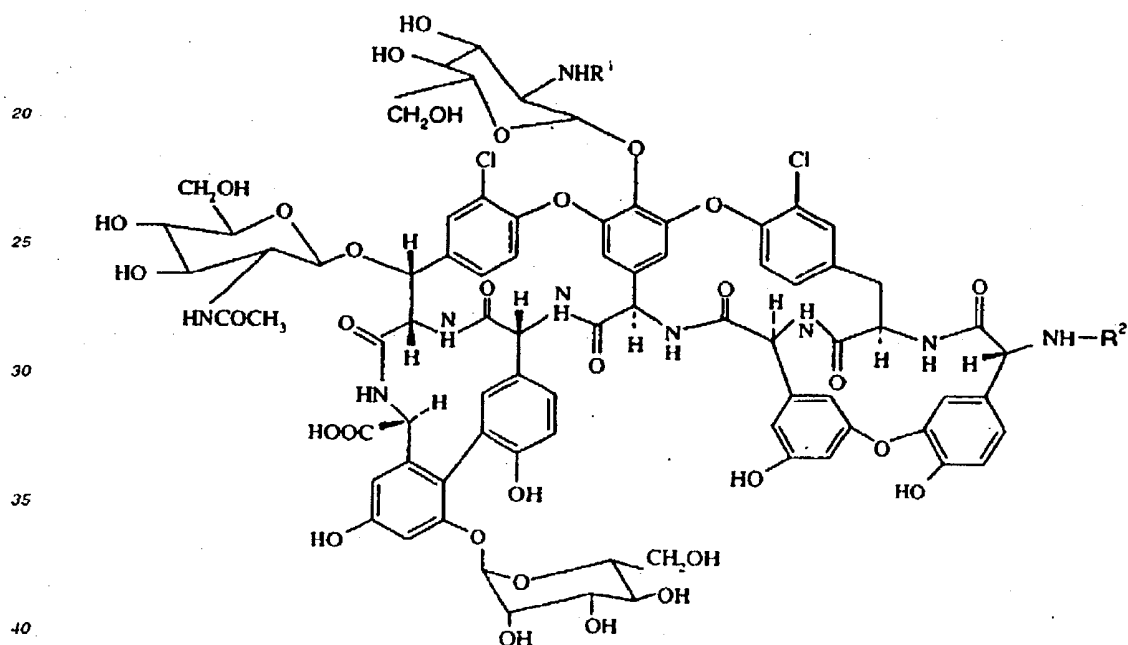
5 This application claims priority of Provisional Application Serial No. 60/042,017, filed April 17, 1997.

BRIEF SUMMARY OF THE INVENTION

10 The present invention is directed to derivatives of teicoplanin, which is an antibiotic of the glycopeptide family, more particularly of the ristocetin type.

DETAILED DESCRIPTION OF THE INVENTION

15 The present invention is directed to compounds of the formula:

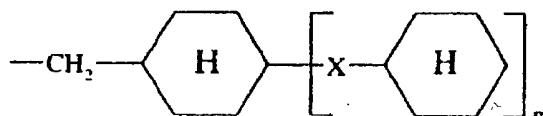


and the pharmaceutically acceptable salts thereof.

20 In the foregoing formula, one of R¹ and R² is:

- alkyl of C₁-C₁₂ wherein the attaching carbon is a CH₂.
- alkenyl of C₂-C₁₂ wherein the attaching carbon is a CH₂.
- alkynyl of C₂-C₁₂ wherein the attaching carbon is a CH₂.

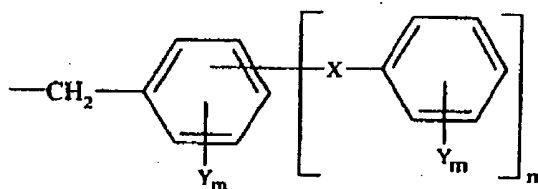
25 cycloalkylmethyl of the formula:



EP 0 873 997 A1

naphthylmethyl,
thienylbenzyl,
phenylthienylmethyl,

benzyl of the formula:



wherein X is a linker of the formula $-(CH_2)_x-Z-(CH_2)_y-$,

wherein each of x and y is 0-6, and the sum of x and y = 0-6, and Z is a bond, -O-, -S-, -CH=CH-, or -C≡C-;

and any Y is independently halo, loweralkyl of C₁-C₅, loweralkoxy of C₁-C₅, loweralkylthio of C₁-C₅, trifluoromethyl, or trifluoromethoxy,

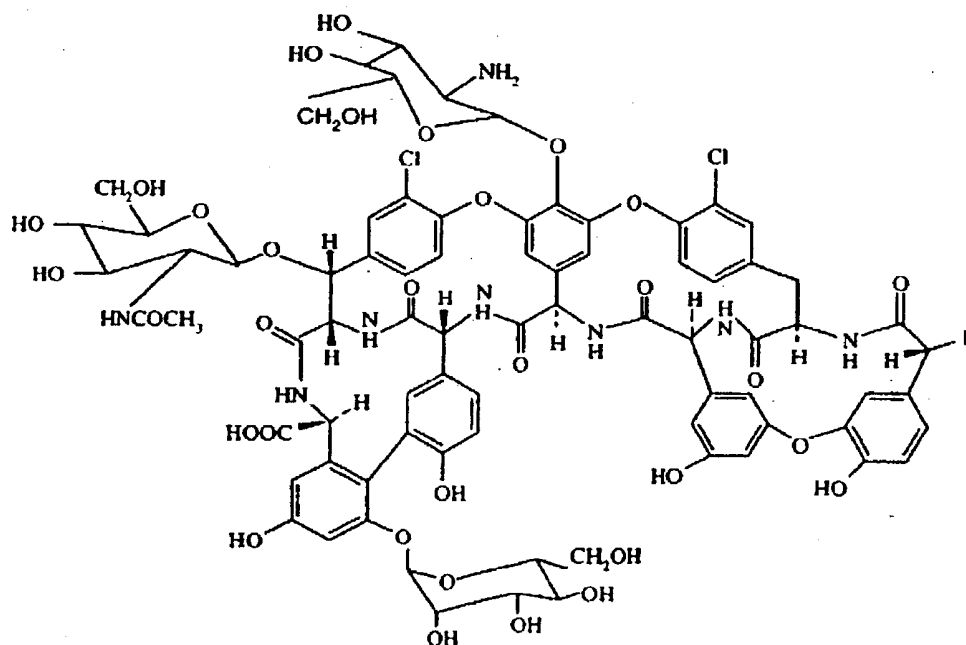
and each m is independently 0 or 1;

and the other of R¹ and R² is identical or is H, or, in the case of R², an amino protecting group.

Any alkyl, alkenyl, or alkynyl can be straight chain or branched.

The foregoing compounds are active as antibiotics, and in particular exhibit activity against gram-positive organisms, including activity against vancomycin-resistant enterococci ("VRE").

The present compounds are prepared by the reaction of deacyl teicoplanin, having the following formula:



wherein R is amino or protected amino. Deacyl teicoplanin is prepared by deacylating teicoplanin. Teicoplanin is a known antibiotic, having five factors differing only as to the identity of the acyl on the amine on the N⁴-sugar. Deacyl teicoplanin is prepared from teicoplanin or teicoplanin wherein the N¹⁵ amine has been protected. Amino protection

EP 0 873 997 A1

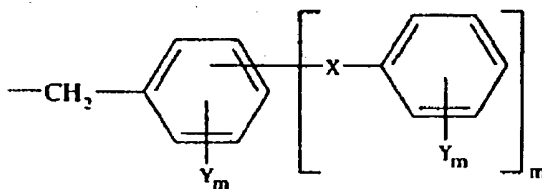
and amino protecting groups are well known. See column 6 of U.S. 5,099,015, which is incorporated herein by reference. Special attention is directed to the references there cited, J.S. Barton, "Protective Groups in Organic Chemistry", J.G.W. McOmie, Ed., Plenum Press, New York, N.Y., 1973, Chapter 2; and T.W. Greene, "Protective Groups in Organic Synthesis", John Wiley and Sons, New York, N.Y., 1981, Chapter 7.

The teicoplanin or N¹⁵-protected teicoplanin is reacted with a deacylating enzyme known as echinocandin B esterase ("ECB esterase"). This reaction is illustrated below in Preparation 1. The production of ECB esterase is described in U.S. Patents 4,293,482 and 4,304,716, which are incorporated herein by reference. Other references regarding ECB deacylase also incorporated herein by reference, include 5,573,936, *Appl. Microbiol. Biotechnol.* (1993) 39:532-536; and international patent application PCT/US95/08832.

The reaction of deacyl teicoplanin to prepare the compounds of the present invention is a reductive alkylation, and is conducted in accordance with known techniques. In general, the reaction consists of reacting deacyl teicoplanin, or a salt thereof, with an aldehyde to form a Schiff's base, which is then reduced to the desired alkylated product. The reducing agent may be added simultaneously with the deacyl teicoplanin and the aldehyde. Whether carried out sequentially or simultaneously, the reaction is carried out in a polar solvent such as DMF, methanol, or a mixture of DMF and methanol and at temperatures of from 25° to 100°C, preferably at temperatures of from 60° to 70°C, employing equimolar amounts or a slight excess of aldehyde. The reducing agent is also preferably employed in excess. A source of soluble copper may be added to the reaction mixture, as illustrated in Examples 2 and 3 below. Copper (II) acetate is a preferred source of copper. The copper is preferably supplied in an amount equimolar with the deacyl teicoplanin.

The compounds of the present invention form salts. These salts are readily prepared by conventional methods well known to those skilled in the art. Pharmaceutically acceptable salts are preferred. Any N¹⁵-protecting group can likewise be removed in conventional methods.

Disubstituted compounds, those wherein R²=R¹, are preferred. Also preferred are compounds where R¹, or both R¹ and R², represent



as defined.

The following examples illustrate the present invention and will enable those skilled in the art to practice the invention.

Preparation 1

Deacyl Teicoplanin

A crude preparation of ECB deacylase determined to contain approximately 15,000 mU of deacylating activity (1.3 liters) was adjusted to pH 7.5 with concentrated ammonium hydroxide. Teicoplanin (1 gram) was added to this enzyme solution maintaining pH at 7.5 with base. Temperature was maintained at 26°C and pH was maintained at 7.5 by adding 1N ammonium hydroxide or 1N hydrochloric acid. After 17 hours under these conditions, HPLC analysis of the reaction mixture indicated that approximately 70% of the teicoplanin was hydrolyzed. HPLC data indicated that additional reaction time under the conditions stated above did not improve yield. The hydrolysis of teicoplanin was monitored by HPLC utilizing a gradient eluent system at 1.0 ml min⁻¹ on a Waters RCM 8x10 RadialPak containing a NovaPak C18 column cartridge with a μBondapak C18 guard column with a detection at 280 nm. Gradient system was as follows: 0.2% aqueous trifluoroacetic acid/ acetonitrile (95/5, v/v) held for 3 minutes, ran 17 minute linear gradient to 0.2% aqueous trifluoroacetic acid/ acetonitrile (60/40, v/v), then ramped down to 0.2% aqueous trifluoroacetic acid/ acetonitrile (95/5, v/v) in 5 minutes. A second 1 gram reaction was conducted as described above.

EXAMPLE 1

Deacyl Teicoplanin, Bis(4-phenylbenzyl) Derivative

A mixture of deacyl teicoplanin trifluoroacetic acid salt, (0.051 g, 0.0295 mmol, 1.0 eq) in DMF (5 mL) under an

EP 0 873 997 A1

atmosphere of argon was treated with 4-biphenylcarboxaldehyde (0.0066 mg, 0.0324 mmol, 1.1 eq.). The resulting mixture was heated to 70° and maintained as such for 2 hours. The solution was then treated with sodium cyanoborohydride (0.0022 mg, 0.0324 mmol, 1.1 eq.). Heating at 70° was continued for an additional 2 hours after which the mixture was stirred at room temperature overnight, diluted with water (25 mL), and lyophilized to give a solid. The solid was purified by preparative reverse-phase high performance liquid chromatography (HPLC) using a Waters 3 X (40 X 100 mm) C18 Nova-Pak cartridge with Waters C18 Nova-pak guard insert and utilizing a TFA buffer system. The TFA system consisted of 15% CH₃CN/0.1% TFA at time zero to 45% CH₃CN/0.1% TFA at 28 min. The analytical method for analysis was accomplished by using a Waters C18 Nova-pak RCM column (8 X 100 mm) with a Nova-pak C18 guard insert, eluting with a 2.0 mL/minute linear gradient of 95% TEAP/5% CH₃CN at time = 0) to 20% TEAP/80% CH₃CN at time = 30 minutes (TEAP = 0.5% aqueous triethylamine adjusted to pH = 3 with phosphoric acid) The fraction containing the product was detected by ultraviolet scan at 235 nm. The organic solvent was removed and the product lyophilized to give the deacyl teicoplanin, bis(4-phenylbenzyl) derivative, as the trifluoroacetic acid salt, a white solid (0.018 g, 30%)

EXAMPLES 2 AND 3

Deacyl Teicoplanin, N¹⁵-(4-phenylbenzyl) Derivative and Deacyl Teicoplanin, N^{9lu}-(4-phenylbenzyl) Derivative

A mixture of deacyl teicoplanin trifluoroacetic acid salt, (0.048 g, 0.0281 mmol, 1.0 eq.), in 1:1 DMF/methanol (4 ml) and copperdiacetate (0.0058 mg, 0.0292 mmol, 1.04 eq.) was stirred together at room temperature for 10 minutes. The solution was then treated with 4-biphenylcarboxaldehyde (0.0061 mg, 0.0337 mmol, 1.2 eq.) and sodium cyanoborohydride (0.0021 mg, 0.0337 mmol, 1.2 eq.) and heated to 70°C for approximately 18 hours. The mixture was concentrated *in vacuo*.

The solid was purified by HPLC as in Example 1 and three products were isolated: (1) monoalkylated product on the N-terminus (R² = 4-phenylbenzyl), as a white solid (0.007 g, 4%); (2) monoalkylated on the glucosamino (R¹ = 4-phenylbenzyl), as a white solid (0.002 g, 4%); and (3) dialkylated product (R¹ = R² = 4-phenylbenzyl) as in Example 1.

EXAMPLE 4

Deacyl Teicoplanin, Bis(10-undecen-1-yl) Derivative

A mixture of deacyl teicoplanin trifluoroacetic acid salt, (0.076 g, 0.0423 mmol, 1.0 eq.), in 6 mL DMF was treated with undecylenic aldehyde (0.016 g, 0.0924 mmol, 2.1 eq.) and sodium cyanoborohydride (0.006 g, 0.0888 mmol, 2.1 eq.). The mixture was stirred at 70° for 2 hours and stirred at room temperature overnight. The mixture was diluted with water (25 mL) and lyophilized to give a solid. The solid was purified by HPLC as in method A. A deacyl teicoplanin, bis(10-undecen-1-yl) derivative product was isolated as the trifluoroacetic acid salt, a white solid (0.0085 g, 9.6%).

Data on the foregoing compounds, and other compounds representative of the present invention, are presented in the following table

TABLE 1

Ex #	R ¹	Method of Ex #	Mass Spec	HPLC Reten. Time	% Yield	R ²
1	4-phenylbenzyl	1	2058 M + 2	9.57	30	=R ¹
2	H	2	1692 M + 3	7.74	13	4-phenylbenzyl
3	4-phenylbenzyl	3	1690 M + 1	9.03	4	H
4	(10-undecen-1-yl)	4	2032 M + 4	12.68	10	=R ¹
5	4-butylbenzyl	1	2018 M + 3	11.26	9	=R ¹
6	2-naphthylmethyl	1	2006 M + 3	10.42	12	=R ¹
7	4-(2-phenylvinyl)-benzyl	1	1917 M + 2	10.47	3	H
8	4-(2-phenylvinyl)-benzyl	1	2109 M + 1	11.82	7	=R ¹
9	4-chlorobenzyl	1	1977 M + 6	9.27	13	=R ¹
10	4-phenoxybenzyl	1	2090 M + 3	10.97	19	=R ¹
11	3-phenoxybenzyl	1	2090 M + 3	10.94	12	=R ¹
12	4-pentyloxybenzyl	1	2080 M + 4	11.96	15	=R ¹

EP 0 873 997 A1

TABLE 1 (continued)

Ex #	R ¹	Method of Ex #	Mass Spec	HPLC Reten. Time	% Yield	R ²
13	4-(trifluoromethoxy)-benzyl	1	2074. M + 3	12.55	14	=R ¹
14	4-(trifluoromethoxy)-benzyl	1	1899. M + 2	10.34	4	H
15	3,5,5-trimethylhexyl	4	1980. M + 4	11.34	3	=R ¹

The present teicoplanin derivatives are useful for the treatment of bacterial infections. Therefore, in another embodiment, the present invention is directed to a method for controlling a bacterial infection in a host animal, typically a warm-blooded animal, which comprises administering to the host animal an effective, antibacterial amount of a compound of the present invention. In this embodiment, the compounds can be used to control and treat infections due to various bacteria, but especially gram-positive bacteria. In a preferred embodiment, the teicoplanin derivatives are used to control and treat infections due to bacteria resistant to existing antibacterials. For example, certain bacteria are resistant to methicillin, and yet others are resistant to vancomycin and/or teicoplanin. The present compounds provide a technique for controlling and treating infections due to such resistant bacterial species.

In carrying out this embodiment of the invention, the compounds can be administered by any of the conventional techniques, including the oral route and parenteral routes such as intravenous and intramuscular. The amount of compound to be employed is not critical and will vary depending on the particular compound employed, the route of administration, the severity of the infection, the interval between dosings, and other factors known to those skilled in the art. In general, a dose of from about 0.5 to about 100 mg/kg will be effective; and in many situations, lesser doses of from about 0.5 to about 50 mg/kg will be effective. A compound of the present invention can be administered in a single dose, but in the known manner of antibacterial therapy, a compound of the present invention is typically administered repeatedly over a period of time, such as a matter of days or weeks, to ensure control of the bacterial infection.

Also in accordance with known antibacterial therapy, a compound of the present invention is typically formulated for convenient delivery of the requisite dose. Therefore, in another embodiment, the present invention is directed to a pharmaceutical formulation comprising a compound of the present invention, in combination with a pharmaceutically-acceptable carrier. Such carriers are well known for both oral and parenteral routes of delivery. In general, a formulation will comprise a compound in a concentration of from about 0.1 to about 90% by weight, and often from about 1.0 to about 3%.

The antibacterial efficacy of the present teicoplanin derivatives is illustrated by the following tables. The minimal inhibitory concentrations (MICs) were determined using a standard broth micro-dilution assay. TABLES 2A, 2B, and 2C present MICs against individual organisms. TABLE 3 presents a comparison of the activity of illustrative compounds against representative vancomycin-resistant and vancomycin-sensitive enterococci (*Enterococcus faecium* and *Enterococcus faecalis*, mean geometric MIC (mcg/mL), as determined by the standard broth micro-dilution assay. In TABLES 2A and 2B "N.G." stands for "no growth", indicating that the microorganism failed to grow in the control

TABLE 2A

Ex #	S. aureus 446	S. aureus 489	S. aureus 447	S. aureus X400	S. aureus X778	S. aureus 491	S. aureus S13E	S. aureus SA1199
1	0.5	0.125	2	0.25	0.25	0.25	0.25	0.25
2	≤03	≤03	0.25	≤03	0.125	≤03	≤03	≤03
4	4	2	8	2	4	4	2	
5	0.5	0.5	0.5	0.25	0.25	0.25	1	0.25
6	≤06	≤06	≤06	≤06	≤06	≤06	≤06	≤06
7	2	0.25	2	0.5	2	1	0.5	
8	1	0.25	1	1	2	1	2	4
9	≤0.06	≤0.06	4	≤0.6	≤0.6	≤0.6	0.25	0.125
10	0.25	0.5	1	0.25	0.5	1	2	2
11	2	1	1	0.5	0.25	0.25	2	0.5
12	4	2	16	2	4	2	2	
13	1	0.5	1	0.5	1	0.5	1	
14	4	2	6	2	4	2	2	
15	1	2	16	2	2	2	2	

EP 0 873 997 A1

TABLE 2B

Ex #	S. aureus SA1199R	S. aureus SA1199B	S. h. 105	S. h. 415	S. cpi 270	E. f. 180	E. f. 180-1	E. f. 2041
1	≤06	0.5	≤06	4	1	≤06	≤06	≤06
2	≤03	≤03	0.25	8	0.25	32	0.25	≤03
4			16	16	2	8		
5	≤06	0.25	0.125	4	0.25	≤06	≤06	≤06
6	≤06	≤06	≤06	4	≤06	0.25	≤06	≤06
7			0.25	32	2	64	1	0.25
8	0.125	2	>64	>64	1	0.25	0.125	0.125
9	≤06	≤06	8	16	0.125	64	≤06	≤06
10	<06	0.5	>64	8	0.5	0.25	<06	<06
11	≤06	0.25	>64	16	≤06	1	0.25	≤06
12			32	64	4	8	N.G.	N.G.
13			32	8	0.5	4	N.G.	N.G.
14			1	>128	2	>128	8	8
15			16	8	4	8	N.G.	N.G.

TABLE 2C

Ex #	E. f. 276	E. gall. 245	M. cat M12	H. influ RD	H. influ RD/ BL+	EC14	St pneu mo P1	St. pyro C203
1	≤06	≤06	≤06	64	64	>64	≤06	≤06
2	<03	0.25	0.25	16	32	>32	<03	<03
4						>128	1	0.5
5	≤06	≤06	≤06	64	1	>64	≤06	≤06
6	≤06	≤06	≤06	16	1	>64	≤06	≤06
7						>128	≤06	0.25
8	0.5	0.25	0.25	N.G.	N.G.	>64	0.25	0.125
9	≤06	≤06	≤06	N.G.	N.G.	>64	≤06	≤06
10	0.5	0.25	≤06	N.G.	N.G.	>64	0.125	≤06
11	0.25	0.125	≤06	N.G.	N.G.	>64	≤06	≤06
12						>128	2	0.5
13						>128	0.05	≤06
14						>128	1	1
15						>128	0.5	0.25

TABLE 3

Ex #	Resist MIC	Sens MIC
1	3.4	0.14
1	0.86	0.046
2	>32	2.6
3	>64	4.6
4	1.2	1.5
5	0.84	0.25
6	3.4	0.11
7	19.03	0.76
8	0.59	0.22
9	13	0.11

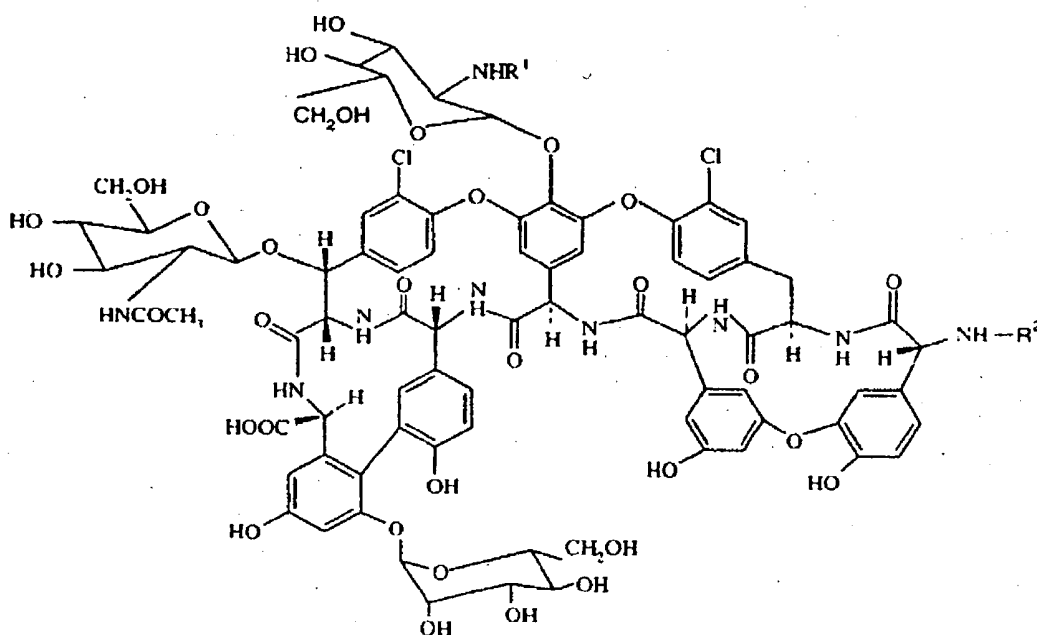
EP 0 873 997 A1

TABLE 3 (continued)

Ex #	Resist. MIC	Sens. MIC
10	1.7	0.19
11	3.4	0.11
12	0.84	0.33
13	45	0.25
14	>32	5.23
15	0.59	0.33

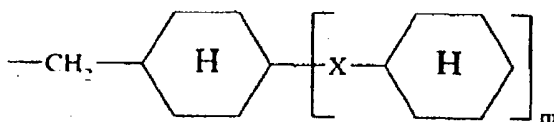
Claims

1. A compound of the formula:



wherein one of R¹ and R² is

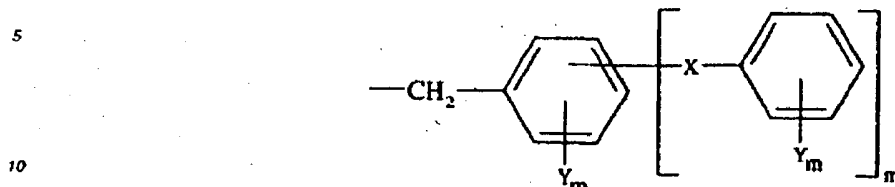
- alkyl of C₁-C₁₂ wherein the attaching carbon is a CH₂,
 alkenyl of C₂-C₁₂ wherein the attaching carbon is a CH₂,
 alkynyl of C₂-C₁₂ wherein the attaching carbon is a CH₂, cycloalkylmethyl of the formula:



naphthylmethyl,
 thienylbenzyl,
 phenylthienylmethyl.

EP 0 873 997 A1

benzyl of the formula:



wherein X is a linker of the formula $-(CH_2)_x-Z-(CH_2)_y-$

wherein each of x and y is 0-6,

and the sum of x and y = 0-6,

and Z is a bond,

-O-,

-S-,

-CH=CH-,

or -C≡C-,

and any Y is independently halo, loweralkyl of C₁-C₅, loweralkoxy of C₁-C₅, loweralkylthio of C₁-C₅,

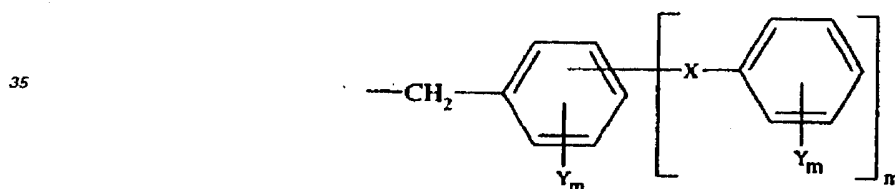
trifluoromethyl,

or trifluoromethoxy,

and each m is independently 0 or 1;

and the other of R¹ and R² is identical or is H, or, in the case of R², an amino protecting group, or a pharmaceutically acceptable salt thereof.

2. A compound of Claim 1 wherein at least one of R¹ or R² is



as defined.

3. A compound of Claim 1 or 2 wherein R¹=R².

4. A pharmaceutical formulation comprising a compound of any of Claims 1-3 in combination with a pharmaceutically acceptable carrier.

5. Use of a formulation of Claim 4 in the manufacture of a medicament for the treatment of a bacterial infection in a host.

6. Use according to Claim 5 in which the bacterial infection is attributable to a vancomycin-resistant-enterococcus.

7. A compound of any of Claim 1-3 for use in antibacterial therapy

8. A compound of any of Claims 1-3 for use in antibacterial therapy against vancomycin-resistant-enterococcus

9. A process for the preparation of a compound of any of Claims 1-3 which comprises reductively alkylating deacyl teicoplanin or an N¹⁵-amino protected teicoplanin, and thereafter optionally forming a pharmaceutically-acceptable salt thereof or removing the amino protecting group, or both forming a salt and removing the amino protecting

EP 0 873 997 A1

group in either order.

5

10

15

20

25

30

35

40

45

50

55

EP 0 873 997 A1

European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 98 30 2868

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	EP 0 306 645 A (GRUPPO LEPETIT) 15 March 1989 * the whole document *	1,4-9	C07K9/00 A61K38/14
X	EP 0 204 179 A (GRUPPO LEPETIT) 10 December 1986 * the whole document *	1,4-9	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
			C07K A61K
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 26 August 1998	Examiner Masturzo, P
CATEGORY OF CITED DOCUMENTS X particularly relevant if taken alone Y particularly relevant if combined with another document of the same category A technological background Q non-written disclosure P prior art document E theory or principle underlying the invention E earlier patent document but published on or after the filing date D document cited in the application L document cited for other reasons S member of the same patent family, corresponding document			

EPO FORM 1503 (02/92) Rev. C/D